

10	20	
1 H T G P G K H K C E C K S H Y V G D G L		BM-HABP Fig.4
1 H M - - - - -		TSG-6.PRO
<hr/>		
30	40	
21 H C E P E E L F I D R C L Q D H G Q C H		BM-HABP Fig.4
3 - - - - - M L L C L - - - - -		TSG-6.PRO
<hr/>		
50	60	
41 A C A R C D L - - - - -		BM-HABP Fig.4
8 - - - - - C V L L V E E A H G S F R N E		TSG-6.PRO
<hr/>		
70	80	
49 H F Q C T - - - - T V G V F H L R S P		BM-HABP Fig.4
24 I F H N S I M P L E I A A G V Y H R E A F		TSG-6.PRO
<hr/>		
90	100	
64 L G Q Y K L T F D K A R E A C A N E E A		BM-HABP Fig.4
44 A G R Y K L T Y A E A K A V C E F E G G		TSG-6.PRO
<hr/>		
110	120	
84 F M A T Y N Q L S Y X Z K A K Y H L C S		BM-HABP Fig.4
64 R L A T Y K Q L E A A R R I G E F H V C A		TSG-6.PRO
<hr/>		
130	140	
104 A S U L E T G R V V A Y P T A F A S Q N C		BM-HABP Fig.4
84 A G U M A K G R V G Y P I V E P G P N C		TSG-6.PRO
<hr/>		
150	160	
124 G S G V V G I V D Y G P R P H K S E M V		BM-HABP Fig.4
104 G F G K T G I T D Y G I R L H R S E R V		TSG-6.PRO
<hr/>		
170	180	
144 D V F C Y R M K D V N C T X - - - - E		BM-HABP Fig.4
124 D A Y C Y H P H A K E C G G V F T D P F		TSG-6.PRO
<hr/>		
190	200	
159 V G Y V G D G F S - - Y S G N L L - -		BM-HABP Fig.4
144 R I E K S P G F P N E Y D D H Q V C Y W		TSG-6.PRO
<hr/>		
210	220	
174 - - - - - V L M S F P S - - - -		BM-HABP Fig.4
164 H I R L K Y G R I H L S F L D F D L E		TSG-6.PRO
<hr/>		
230	240	
182 - - - - - L T N F L T E V L A Y S N S S		BM-HABP Fig.4
184 H D P G C L A D Y V E I Y D S Y D D V H		TSG-6.PRO
<hr/>		
250	260	
197 A R G R A F L E H L T D L S I R G T L F		BM-HABP Fig.4
204 G - - - - - - - - - - - - - - - E		TSG-6.PRO
<hr/>		
270	280	
217 V P I - - N S G L G E N E T L S G R D I		BM-HABP Fig.4
206 V S R Y C G D E L P E D I F S T G H V M		TSG-6.PRO
<hr/>		
290	300	
235 E H H L A N V S M F F Y N D L V N G T T		BM-HABP Fig.4
226 F L H F - - - - - - - - - L S C D A S V		TSG-6.PRO
<hr/>		
310	320	
255 L Q T R L G S K L L I T D F Q D P L H F		BM-HABP Fig.4
236 T A S S F Q I F Y W T V D - - - - -		TSG-6.PRO
<hr/>		
330	340	
275 T E T R C V D S R D T L E V D I C A S N		BM-HABP Fig.4
249 P A S K E S Q A K N T - - - - - S T T		TSG-6.PRO
<hr/>		
350	360	
295 G I T H V I S F X L K A P P A P V T L X		BM-HABP Fig.4
263 G N K E F L P - - - - - - - - -		TSG-6.PRO
<hr/>		
370	380	
315 H T G L S X G I F X X K I L V T G A V A		BM-HABP Fig.4
270 - - - - - - - - - - - - - - -		TSG-6.PRO
<hr/>		
390		
335 L A A V S Y F R I N R R T I G S F X H F		BM-HABP Fig.4
270 - G R F S H L .		TSG-6.PRO

Decoration 'Decoration #1': Shade (with solid black) residues that match the Consensus exactly.

Divergence

Percent Identity

	1	2	
1		31.6	1
2	100.0		2
	1	2	

BM-HABP Fig.4.PRO

TSG-6.PRO



Blast 2 Sequences results

[PubMed](#)[Entrez](#)[BLAST](#)[OMIM](#)[Taxonomy](#)[Structure](#)

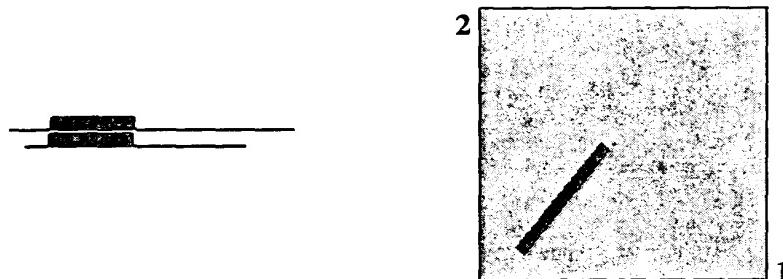
BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.1.2 [Oct-19-2000]

Matrix **BLOSUM62** gap open: **11** gap extension: **1**

x_dropoff: **50** expect: **10.000** wordsize: **3** Filter Align

Sequence 1 lclseq_1 Length 353 (1 .. 353)

Sequence 2 lclseq_2 Length 275 (1 .. 275)



NOTE: The statistics (bitscore and expect value) is calculated based on the size of nr database

Score = 107 bits (264), Expect = 5e-21
Identities = 45/104 (43%), Positives = 61/104 (58%)

Query: 52 DTTVGVFHLRSPLGQYKLTFDKAREACANEAAATMATYNQLSYXQAKYHLCAGWLETGR 111
+ GV+H + G+YKLT+ +A+ C E +ATY QL +K +H+C+AGW+ GR
Sbjct: 32 EQAAGVYHREARAGRYKLTYAEAKAVCEFEGGRLATYKQLEAARKIGFHVCAGWMAKGR 91

Query: 112 VAYPTAFASQNCGSGVVGIVDYGPRPNKSEMWDVFCYRMKDVCN 155
V YP NCG G GI+DYG R N+SE WD +CY C
Sbjct: 92 VGYPPIVKPGPNCGFGKTGIIDYGIRLNRSERWDAYCYNPHAKEC 135

CPU time: 0.15 user secs. 0.03 sys. secs 0.18 total secs.

Gapped
Lambda K H
0.321 0.138 0.429

Gapped
Lambda K H
0.270 0.0470 0.230

Matrix: BLOSUM62
Gap Penalties: Existence: 11, Extension: 1
Number of Hits to DB: 748
Number of Sequences: 0
Number of extensions: 55
Number of successful extensions: 1
Number of sequences better than 10.0: 1
Number of HSP's better than 10.0 without gapping: 1
Number of HSP's successfully gapped in prelim test: 0
Number of HSP's that attempted gapping in prelim test: 0
Number of HSP's gapped (non-prelim): 1

length of query: 353
length of database: 3,171,650,076
effective HSP length: 58
effective length of query: 295
effective length of database: 3,171,650,018
effective search space: 935636755310
effective search space used: 935636755310
T: 9
A: 40
X1: 16 (7.4 bits)
X2: 128 (49.9 bits)
X3: 128 (49.9 bits)
S1: 41 (21.9 bits)
S2: 83 (36.7 bits)

EXHIBIT C

56 GVFHLRSPLGQYKLTDFKAREACANEAAATMATYNQLSYXQAKYHLCAGWLETGRVAYP -**SEQ ID NO:11**
1063 GVFHLRSPLGQYKLTDFKAREACANEAAATMATYNQLSYA**QAKYHLCAGWLETGRVAYP -human HARE**

115 TAFASQNCGSGVVGIVDYGPRPNKSEMWDVFCYR 149
1122 TAFASQNCGSGVVGIVDYGPRPNKSEMWDVFCYR 1156

CURRENT PROTOCOLS IN MOLECULAR BIOLOGY

VOLUME 1

EDITORIAL BOARD

Frederick M. Ausubel
Massachusetts General Hospital & Harvard Medical School

Roger Brent
Massachusetts General Hospital & Harvard Medical School

Robert E. Kingston
Massachusetts General Hospital & Harvard Medical School

David D. Moore
Massachusetts General Hospital & Harvard Medical School

J.G. Seidman
Harvard Medical School

John A. Smith
University of Alabama

Kevin Struhl
Harvard Medical School

GUEST EDITORS

Lisa M. Albright
DNA Sequencing

Donald M. Coen
Harvard Medical School
Polymerase Chain Reaction

Ajit Varki
University of California San Diego
Glycoproteins

SERIES EDITOR
Virginia Benson Chanda



John Wiley & Sons, Inc.

CORE 13 (S15)

Copyright © 1994-1997 by John Wiley & Sons, Inc.

Copyright © 1987-1994 by Current Protocols

All rights reserved. Published simultaneously in Canada.

Reproduction or translation of any part of this work beyond that permitted by Section 107 or 108 of the 1976 United States Copyright Act without the permission of the copyright owner is unlawful. Requests for permission or further information should be addressed to the Permissions Department, John Wiley & Sons, Inc.

While the authors, editors, and publisher believe that the specification and usage of reagents, equipment, and devices, as set forth in this book, are in accord with current recommendations and practice at the time of publication, they accept no legal responsibility for any errors or omissions, and make no warranty, express or implied, with respect to material contained herein. In view of ongoing research, equipment modifications, changes in governmental regulations, and the constant flow of information relating to the use of experimental reagents, equipment, and devices, the reader is urged to review and evaluate the information provided in the package insert or instructions for each chemical, piece of equipment, reagent, or device for, among other things, any changes in the instructions or indication of usage and for added warnings and precautions. This is particularly important in regard to new or infrequently employed chemicals or experimental reagents.

Library of Congress Cataloging in Publication Data:

Current protocols in molecular biology. 3 vols.

1. Molecular biology—Technique. 2. Molecular biology—Laboratory
mammals. 1. Ausubel, Frederick M.

QH506.C87 1987 574.8'7'023 87-21033
ISBN 0-471-50338-X

Printed in the United States of America

20 19 18 17 16 15 14 13

- 1c. *Harsh treatment:* Pour several hundred milliliters of boiling 0.1% SDS onto the membrane. Cool to room temperature.
- If a membrane is to be reprobed, it must not be allowed to dry out between hybridization and stripping. If it becomes dry, the probe may bind to the matrix.*
2. Place membrane on a sheet of dry Whatman 3MM filter paper and blot excess liquid with a second sheet. Wrap the membrane in plastic wrap and set up an autoradiograph.
- If signal is still seen after autoradiography, rewash using harsher conditions.*
3. The membrane can now be rehybridized. Alternatively, it can be dried and stored for later use.
- Membranes can be stored dry between Whatman 3MM paper for several months at room temperature. For long-term storage, place the membranes in a desiccator at room temperature or 4°C.*

REAGENTS AND SOLUTIONS

Aqueous prehybridization/hybridization (APH) solution

5× SSC (APPENDIX 2)

5× Denhardt solution (APPENDIX 2)

1% (w/v) SDS

Add 100 µg/ml denatured salmon sperm DNA (see below) just before use

Alternatives to Denhardt solution and denatured salmon sperm DNA as blocking agents are listed in Table 2.10.5 (see discussion in critical parameters).

Denatured salmon sperm DNA

Dissolve 10 mg Sigma type III salmon sperm DNA (sodium salt) in 1 ml water. Pass vigorously through a 17-G needle 20 times to shear the DNA. Place in a boiling water bath for 10 min, then chill. Use immediately or store at -20°C in small aliquots. If stored, reheat to 100°C for 5 min and chill on ice immediately before using.

Formamide prehybridization/hybridization (FPH) solution

5× SSC (APPENDIX 2)

5× Denhardt solution (APPENDIX 2)

50% (w/v) formamide

1% (w/v) SDS

Add 100 µg/ml denatured salmon sperm DNA (see above) just before use

Alternatives to Denhardt solution and denatured salmon sperm DNA as blocking agents are listed in Table 2.10.5 (see discussion in critical parameters).

Commercial formamide is usually satisfactory for use. If the liquid has a yellow color, deionize as follows: add 5 g of mixed-bed ion-exchange resin [e.g., Bio-Rad AG 501-X8 or 501-X8(D) resins] per 100 ml formamide, stir at room temperature for 1 hr, and filter through Whatman no. 1 paper.

CAUTION: Formamide is a teratogen. Handle with care.

Labeling buffer

200 mM Tris-Cl, pH 7.5

30 mM MgCl₂

10 mM spermidine

Mild stripping solution

5 mM Tris-Cl, pH 8.0

2 mM EDTA

0.1× Denhardt solution (APPENDIX 2)

SDS electrophoresis buffer, 5×

15.1 g Tris base

72.0 g glycine

5.0 g SDS

H₂O to 1000 ml

Dilute to 1× or 2× for working solution, as appropriate

Do not adjust the pH of the stock solution, as the solution is pH 8.3 when diluted. Store at 0° to 4°C until use (up to 1 month).

SED (standard enzyme diluent)

20 mM Tris-Cl, pH 7.5

500 µg/ml bovine serum albumin (Pentax Fraction V)

10 mM 2-mercaptoethanol

Store up to 1 month at 4°C

Sodium acetate, 3 M

Dissolve 408 g sodium acetate·3H₂O in 800 ml H₂O

Add H₂O to 1 liter

Adjust pH to 4.8 or 5.2 (as desired) with 3 M acetic acid

Sodium acetate buffer, 0.1 M

Solution A: 11.55 ml glacial acetic acid/liter (0.2 M).

Solution B: 27.2 g sodium acetate (NaC₂H₃O₂·3H₂O)/liter (0.2 M).

Referring to Table A.2.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H₂O to 100 ml. (See Potassium acetate buffer recipe for further details.)

Sodium phosphate buffer, 0.1 M

Solution A: 27.6 g NaH₂PO₄·H₂O per liter (0.2 M).

Solution B: 53.65 g Na₂HPO₄·7H₂O per liter (0.2 M).

Referring to Table A.2.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H₂O to 200 ml. (See Potassium phosphate buffer recipe for further details.)

SSC (sodium chloride/sodium citrate), 20×

3 M NaCl (175 g/liter)

0.3 M Na₂citrate·2H₂O (88 g/liter)

Adjust pH to 7.0 with 1 M HCl

STE buffer

10 mM Tris-Cl, pH 7.5

10 mM NaCl

1 mM EDTA, pH 8.0

TAE (Tris/acetate/EDTA) electrophoresis buffer

50× stock solution:

242 g Tris base

57.1 ml glacial acetic acid

37.2 g Na₂EDTA·2H₂O

H₂O to 1 liter

Working solution, pH ~8.5:

40 mM Tris-acetate

2 mM Na₂EDTA·2H₂O

TBE (Tris/borate/EDTA) electrophoresis buffer

10× stock solution, 1 liter:

108 g Tris base (890 mM)

55 g boric acid (890 mM)

40 ml 0.5 M EDTA, pH 8.0 (20 mM)